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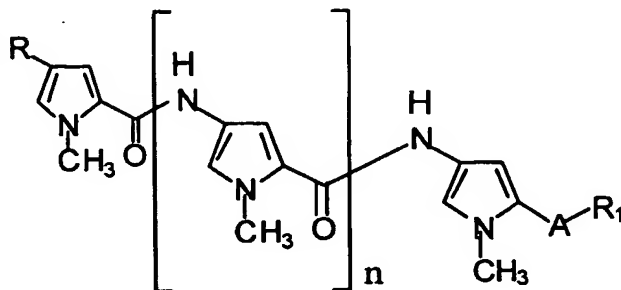
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(1)

(57) Abstract: Use of distamycin, analogues and/or  
derivatives thereof in the manufacture of a pharmaceu-  
tical composition having activity against endoparasito-  
sis in animals.

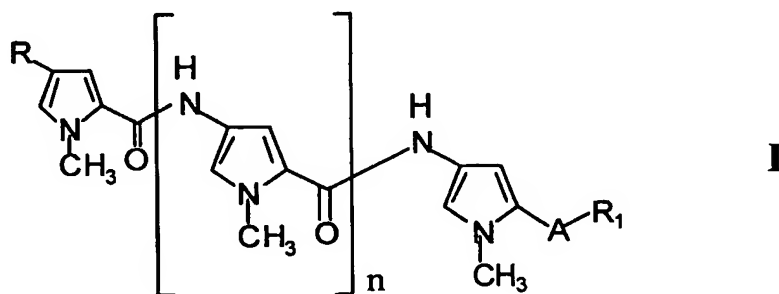
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USE OF POLY-AMINOPYRROLECARBOXAMIDES ALONE OR IN COMBINATION WITH A CYCLODEXTRIN  
IN THE PROPHYLAXIS AND TREATMENT OF ANIMAL PARASITOSIS

### Field of the invention

5 The present invention relates to the use of a compound having the following formula

(I):



wherein:

n is 0 or an integer comprised between 1 and 5;

10 R is a group  $R_2-X-C(=Z)-NH-$ , in which X represents a simple chemical bond, an aromatic or heteroaromatic radical, Z represents an oxygen atom or the NH group; and:

if X is a simple chemical bond,  $R_2$  is an oxygen atom, an alkyl, dialkylaminoalkyl, alkenyl, cycloalkyl, arylalkyl, arylalkenyl, haloalkyl, or an aromatic or heteroaromatic radical;

15 if X is an aromatic or heteroaromatic radical,  $R_2$  is nitro, amino or formylamino;

or:

R is a group  $R_3-C(=Z)-$ , in which Z represents an oxygen atom or the NH group, and  $R_3$  represents a hydrogen atom, the  $-OR_4$  or  $-NR_5R_6$  group, and where:

$R_4$  is chosen from the group consisting of a hydrogen atom, an alkyl, cycloalkyl, arylalkyl, or an aromatic radical;

20

R<sub>5</sub> and R<sub>6</sub>, either the same or different, are chosen from the group consisting of a hydrogen atom, an alkyl, cycloalkyl, arylalkyl, aromatic or heterocyclic radical, optionally substituted with a formylamino or a carbamoyl group; or

5 R<sub>5</sub> and R<sub>6</sub>, joined together form an alkylene group, or the group -(CH<sub>2</sub>)<sub>2</sub>-O-(CH<sub>2</sub>)<sub>2</sub>- or the group -(CH<sub>2</sub>)<sub>2</sub>-NH-(CH<sub>2</sub>)<sub>2</sub>-;

A represents a simple chemical bond or the group -CO-NH-Y-, wherein Y is an alkylene or aromatic radical;

R<sub>1</sub> is chosen from the group consisting of -COOR<sub>4</sub>, -B-NR<sub>5</sub>R<sub>6</sub>, -C(=NH)-NH<sub>2</sub>, a heterocyclic radical containing nitrogen, wherein:

10 R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are as defined above, B represents a simple chemical bond or the -C=O group, and:

when R<sub>1</sub> is -B-NR<sub>5</sub>R<sub>6</sub>, and B is a simple chemical bond, or when R<sub>1</sub> is a heterocyclic radical, A is not a chemical bond;

or a pharmaceutical acceptable salt thereof in the manufacture of a pharmaceutical composition having activity against endoparasitosis in animals.

15 When: n is 1, and R represents the group R<sub>2</sub>-X -C(=Z)-NH-, in which X is a chemical bond, R<sub>2</sub> is hydrogen and Z is oxygen, and A represents the group -CO-NH-Y, in which Y is -CH<sub>2</sub>-CH<sub>2</sub>-, and R<sub>1</sub> represents the group -C(=NH)-NH<sub>2</sub>, the compound of formula (I) is the well known antiviral antibiotic Distamycin, isolated from and produced  
20 by the mycelium of *Streptomyces distallicus* [A.M. Casazza et al., Antimicrobial Agents Chemother, 5:593 (1965)]. Compounds of general formula (I) are known compounds the preparation of which has been disclosed [ F. Arcamone, et al., Gazz. Chim. Ital. 97:1110 (1967); G. DiPietro et al., Perkin Comm., 1996:1333; P. Lombardi et al., Pharmacol. Ther., 76:125 (1997); F. Animati et al., J. Med. Chem., 38:1140 (1995); WO 9209574; US  
25 5472976; US 5670534]. For the compounds of formula (I) there are generically mentioned

various pharmacological activities, e. g. antiviral and antimalarial activities, but said compounds have not found use in the clinical practice. In the previous art, however, there are no teachings of a specific activity of the compounds of the invention in the treatment of endoparasitosis in animals or of their use in veterinary medicine.

5       The pharmaceutical compositions according to the invention comprise a compound of formula (I), or a pharmaceutical acceptable salt thereof, as an active principle, and a pharmaceutical acceptable diluent and/or carrier.

      An object of the present invention is in particular the use of a compound of formula (I), or a pharmaceutical acceptable salt thereof, in association or combination with a  
10   cyclodextrin in the manufacture of a veterinary pharmaceutical composition having activity against endoparasitosis in animals.

#### Background of the invention

      It would be highly desirable to accomplish further progress in order to achieve more efficient treatments, free from undue side effects, against the numerous animal  
15   parasitoses. At present, there are as yet no reliable therapies for many of those, due to lack of specific drugs; development of resistance to current drugs and of immunodepression in the hosts; widespread resistance of parasites to different environmental conditions, including chemicals usually employed as disinfectants.

      Parasites are pathogenic agents by definition, perpetually causing to the host  
20   damages which may become more or less severe in relation to the pathogenicity of parasites, their prevalence and the susceptibility of the host, resulting in the onset of diseases which are very often chronic. Moreover, parasites favour the occurrence of other pathogenic agents resulting in more severe conditions, since modifications caused by the parasite may assist infections by other pathogenic microorganisms.

In the case of production or farm animals, this results in reduced yield and quality of products (such as milk, meat, wool and their derivatives) causing significant economic losses to the rearing and production markets, and potential health implications for consumers.

5       Accordingly, in recent years parasitic infections are major problems in zootechnics, since parasitic infections have become emerging pathologies as a consequence of new production improvement techniques, modern farm management, and breeds of animal with better track records in productivity. In these conditions, the opportunistic nature typical of parasites finds the most favourable conditions to expand its pathogenic potential since the  
10       rate of subject-to-subject transmission (particularly high in the confinement rearing which determines a large number of animals permanently housed in restricted spaces) and the decrease of the specific immune responses of the host (bound to a series of factors which characterize intensive farming) allow for the rapid replication and spread of the parasite, resulting in very high prevalences of infection in affected farms, turning into a perpetual  
15       threat to animal health and productivity.

      In this context, endoparasitoses and, particularly, parasitoses associated with unicellular enteropathogenic parasites, are object of major interest in the medical veterinary practice and research, both for infectivity, morbidity and mortality characteristics in different hosts and for the wide diffusion and capacity to sustain severe zoonotic  
20       infections which may result in the death of immunocompromised subjects. Moreover, for many causative agents, the lack of host-specificity determines numerous, actually uncontrollable routes of transmission with respect both to animals and humans. The same parasitic species can be detected in tens of farm, companion or wild animal species, and world-wide.

25       Important or emerging parasitic pathologies are, for example:

Trichomoniasis, which afflicts birds (turkeys, chickens, etc.) caused by *T. gallinae* and *T. gallinarum*, and infections by *Hexamita meleagridis*, which colonize the upper part of the digestive tract and the distal part of the intestine;

5 Giardiasis, found world-wide and potentially present in all animals, caused by parasites of which the most common are *G. lamblia* or *G. enterica*, *G. canis*, *G. cati*, *G. bovis*, *G. duodenalis*, *G. chinchillae* (particularly severe in chinchilla farms), *G. intestinalis*, localized in the duodenum and colon;

Istomoniasis, or infectious enteropathitis, caused by *Histomonas meleagridis*, localized in the coecum of birds, particularly of turkeys;

10 Amoebiasis, caused by parasites of the genus *Entamoeba*, such as for instance *E. histolytica*, localized in the large intestine (coecum and colon), which particularly afflicts humans, but which may be frequently present in animals, which are reservoirs of infection for humans;

Coccidiosis, which can develop in almost all domestic and wild animals, and even in  
15 humans, found world-wide and also endemically, caused by families of numerous intestinal parasites, such as species belonging to the genus *Eimeria*, for example, *E. bovis*, *E. ellipsoidalis*, *E. necatrix*, *E. tenella*, *E. stiedae*, *E. alabamensis*, *E. perforans*, and *E. zuernii*; species belonging to the genus *Isospora*, for example, *I. hominis*, *I. belli*, *I. bigemina*, *I. rivolta*, *I. felis*; species belonging to the genus *Cryptosporidium*, for example,  
20 *C. parvum*, *C. muris*, *C. bailey*, *C. felis*, *C. canis*, *C. meleagridis*, *C. nasorum*, *C. serpentis*, *C. wrairi*, *C. andersoni*, *C. blagburni*, *C. saurophilum*, *C. molnari*;

Balantidiosis, caused by *Balantidium coli*, localized in the large intestine of pigs, but also of cattle and humans.

As a consequence of present investigation techniques, new aspects concerning the  
25 genetic polymorphism of the above mentioned parasites are continuously elucidated and new

classifications are set forth, therefore the above mentioned parasitic species are only given for purpose of exemplification and their number is subjected to increase.

Although many chemotherapeutic compounds have been tested for efficacy against enteropathogenic parasites in animals, there is no clearly recognized, widely accepted, or immediately available compounds for prophylaxis or therapy. Some drugs have demonstrated to improve in part the symptomatology and rate of mortality within a farm, others turned to be toxic to the animal at the therapeutically effective dose.

#### Detailed description of the invention

It has been found that the compounds of formula (I) according to the present invention, besides exhibiting antiviral and antimalarial activities, surprisingly exhibit at various degrees, and according to the different substituents, in vitro and in vivo antiparasitic activities with respect to internal parasites which afflict animals, in particular with respect to parasites located in the gastro-intestinal tract (enteropathogenic or intestinal parasites), which are the causative agents of several, and sometimes severe, enteric infections and enteritis, as mentioned herebefore.

The water solubility of the pharmaceutically acceptable salts of the compounds of formula (I) and the surprising absence of oral bioavailability, which renders the inventive compounds substantially safe and non-toxic with respect to the recipient animal, allow the compounds of the invention to achieve the required therapeutic and prophylactic action against the above-mentioned parasites in the cases both of acute infection and when there is a need to keep the parasite population under a safe control.

It is known, in fact, that affected animals often reject food, but very rarely refuse to drink. It is also known that several drugs used in the veterinary practice, and particularly drugs used to control parasites, may be found as such, or as metabolites thereof, in the animal products for human use (meat, milk).

Therefore, the compounds of the present invention exhibit a remarkable useful advantage with respect to many drugs of the prior art in that they can be administered as dissolved in drinking water and, not been adsorbed across the gastrointestinal tract when administered to the patient orally, they neither diffuse in the animal tissues nor are here  
5 metabolized.

The pharmaceutical acceptable salts of the compounds of formula (I) are the salts by addition of pharmaceutically acceptable inorganic acids, such as for instance hydrochloric, sulphuric, phosphoric acid and the like, as well of pharmaceutically acceptable organic acids, such as for instance acetic, maleic, ascorbic, succinic, benzoic, salicylic acid, and the  
10 like. Particularly preferred are the salts by addition of hydrochloric acid.

Preferred examples of the compounds according to the present invention are:

distamycin; and the compounds of formula (I) wherein:

n is as previously defined;

R is the  $-\text{CONH}_2$  group, A is the  $-\text{CONHCH}_2\text{CH}_2-$  group,  $\text{R}_1$  is the  $-\text{C}(=\text{NH})-$   
15  $\text{NH}_2$  group or the  $-\text{CH}_2\text{N}(\text{CH}_3)_2$  group;

R is the  $-\text{NH}-\text{CH}(=\text{NH})$  group, A is the  $-\text{CONHCH}_2\text{CH}_2-$  group,  $\text{R}_1$  is the  
-  $\text{C}(=\text{NH})-\text{NH}_2$  group or the  $-\text{CH}_2\text{N}(\text{CH}_3)_2$  group or the  $-\text{CONH}_2$  group;

and the pharmaceutically acceptable salts thereof.

The compounds of formula (I) appear not to be orally bioavailable, as set forth in the  
20 Example 3, where for the only purpose of exemplification but not of limitation, there is presented a study comparing blood concentrations of a compound of the invention in the rat following intravenous (i. v.) and oral (p. o.) administration, respectively.

The pharmaceutical composition of the present invention, therefore, finds useful application in the prophylaxis and therapeutic treatment of endoparasitosis in animals, in  
25 particular production and farm animals, including mammals, such as cattle, sheep, goats,



pigs and horses, and non-mammals, such as chickens, turkeys, pigeons, ducks, geese, quails, pheasants, as well as companion pets, such as dogs, cats, and cage birds.

Preferred examples of parasite-caused enteric diseases which can be prevented or treated with the pharmaceutical composition of the invention are Trichomoniasis, Giardiasis, Istomoniasis, Amoebiasis, Coccidiosis, and Balantidiosis.

The pharmaceutical composition of the present invention is preferably administered orally to the animal, for instance as dissolved in drinking water or admixed with food.

A further object of the present invention is a method of treatment of endoparasitosis in an animal, in need of such a treatment, comprising administering by oral route to said animal a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof.

The compounds according to the invention can be administered to animals orally in various solid or non-solid forms, such as, for instance, tablets, capsules, tablets coated with films or sugar, solutions or suspensions, preferably as solutions, preferably dissolved in water.

The dosage is dependent on the age, weight, general condition, species of the animal and on the route of administration, for instance by bolus, dispersion in the drinking water or in the food. For example, an effective dosage for an adult animal can be comprised between 0.1 and 500 mg/kg pro dose administered from 1 to 5 times per day, or can be a concentration comprised between 0,5 and 5 g/L drinking water or can be an amount comprised between 1 and 10 g/kg food, according to the judgement of the attending veterinary physician.

Pharmaceutical compositions comprising the compounds of the invention are prepared according to conventional techniques. Solid dosage forms may contain, for instance, in addition to the active principle, diluents such as lactose, dextrose, sucrose,

cellulose, potato or cereal starches; lubricants such as silicium oxide, talc, stearic acid, calcium stearate, magnesium stearate and/or polyethylene glycols; binders such as starches, arabic gum, gelatin, methylcellulose, carboxymethylcellulose or polyvinylpyrrolidinone; disintegrating agents such as starch, alginic acid, alginates and amides, sodium glycolate; 5 dyes; sweeteners; wetting agents such as lecithin, polysorbates, laurylsulphates; and generally non-toxic and pharmaceutically inactive substances such as those conventionally used in pharmaceutical formulations. Such pharmaceutical preparations can be made up according to known methods, e. g. by mixing, grinding, tableting, coating processes. Liquid dispersions may be for instance syrups, solutions, emulsions and suspensions. 10 Syrups and solutions may contain carriers such as sucrose, glycerol, mannitol, sorbitol. Suspensions and emulsions may contain carriers such as a gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, polyvinyl alcohol.

A further specific object of the present invention is also a pharmaceutical composition comprising a compound according to the invention in association and/or 15 combination with a cyclodextrin. Cyclodextrins (hereinafter CD) are well known cyclic oligosaccharides, made up of D-glucose residues, having a cylindrical cavity shaped structure capable of including various molecules (guest molecules). One of the most interesting properties of CD is their ability to form inclusion compounds or complexes. This kind of complexation confers new physicochemical properties to guest molecules, and 20 is extensively used and exploited in the pharmaceutical field to improve the solubility and stability of pharmacologically active substances (O. Beckers et al., Drug Dev. Ind. Pharm., 17:1503 (1991); J. Szejtli, Pharm. Tech. Int., February 1991: 15) and, accordingly, their dissolution characteristics and bioavailability.

Said inclusion complexes are usually prepared in a liquid medium, such as water or 25 mixtures of water and organic solvents, and are obtained as powders upon drying.

A variety of methods are known in the art for preparing solid inclusion compounds, such as kneading (K. Uekama et al., *Int. J. Pharm.*, 10:1 (1982)); co-precipitation (K. Uekama et al., *Int. J. Pharm.*, 16:327 (1983)); spray-drying (H.P.R. Bootsma et al., *Int. J. Pharm.*, 51:213 (1989); freeze-drying (US Pat. No. 4,603,123). In some cases the  
5 formation of the complex in the solid phase is thermodynamically spontaneous and inclusion could be achieved by simply grinding (C. Torricelli et al., *Int. J. Pharm.*, 71:19 (1991)).

Moreover, it is known that the effectivity of certain drugs can be still improved with CD also for molecules which are highly soluble in water (such as the pharmaceutically  
10 acceptable salts of the compounds of the invention), that theoretically do not need any particular formulation intended for improving their solubility or their rate of dissolution (US Pat. No. 5,712,260).

In the instant object, the general terms "cyclodextrin" and "CD" refers to naturally-occurring ( $\alpha$ -CD,  $\beta$ -CD and  $\gamma$ -CD), synthetic or semi-synthetic (e. g. hydroxypropyl- $\beta$ -CD  
15 or dimethyl- $\beta$ -CD) cyclodextrins. Particularly preferred CDs are  $\beta$ -cyclodextrin, hydroxypropyl- $\beta$ -cyclodextrin, and  $\gamma$ -cyclodextrin.

It has been surprisingly found that when the pharmaceutically acceptable salts of the compounds of formula (I) are mixed with a cyclodextrin, the solubility of the compounds according to the invention does not change significantly, but the effects of reprecipitation  
20 induced by ionic species or pH changes on the compounds of formula (I) are negligible, since cyclodextrins are able to mask the site of interaction of the compounds of formula (I).

Thus, the formation of a complex in solution between the compounds of the invention and an appropriate cyclodextrin may avoid the incidental reprecipitation of the compounds of the invention either as free bases or as insoluble salts in physiological  
25 conditions. This phenomenon can result in a big advantage in the administration of the

compounds according to invention by oral route, because their antiparasitic efficacy is not affected by the environment of the gastroenteric tract (stomach, bowel) and by their particular conditions (pH, presence of ionic species which may render the compounds of the invention insoluble, fasting/non-fasting conditions of the subject animal during  
5 treatment).

Moreover, it has been noticed that it is not necessary to form the complex between a compound of formula (I) and a CD in the solid state, but it is sufficient to administer a simple physical mixture of the two chemical entities, i. e., a combination or association thereof.

10 The proportion between a compound of formula (I) and a CD may vary, e. g., from 1:0,5 to 1:10 as molar ratio, preferably from 1:1 to 1:4.

It is therefore an object of the present invention a pharmaceutical formulation comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, in association and/or combination with a CD, which can be prepared following known and  
15 conventional procedures.

It is a further specific object of the instant invention a method of treatment of endoparasitosis in animals comprising the oral administration of a composition containing a therapeutically efficient amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof, in association and/or combination with a CD, to an animal in need  
20 of such a treatment.

The following Examples are only intended to illustrate the invention without limiting the same.

#### Example 1

In vitro activity. CACO2 cell cultures ( $2 \times 10^6$  cells 70% confluent) were inoculated  
25 with purified sporozoites of *C. parvum* and, at the time of infection, treated with either

variable concentrations of hydrochloride of the compound of formula (I,  $R = -COONH_2$ ,  $A = -CONHCH_2CH_2-$ ,  $R_1 = -C(=NH)-NH_2$ ) dissolved in dimethylsulphoxide (DMSO) or DMSO (5% medium) in controls. The test compound was removed 30 minutes later by repeated washings. The percentage of infected cells was determined 24 hours after beginning of experiment by microscopic examination after Giemsa staining, analysing 10 microscopic fields for each experimental point at 400x. The values obtained for each point represent the mean of three independent replicates. The percentage of infected cells in untreated controls (i. e., treated with DMSO only) was 7.1% for Experiment 1, and 6.35 for experiment 2. Results set forth in Figure 1 show a remarkable cytotoxic activity of the test compound against the object parasite.

### Example 2

In vivo activity. The in vivo oral antiparasitic activity of the compound of formula (I,  $R = -COONH_2$ ,  $A = -CONHCH_2CH_2-$ ,  $R_1 = -C(=NH)-NH_2$ ) was assessed by evaluating the ability of test compound to modify the course of experimentally induced infection in immuno-suppressed BALB/C mice. After several weeks of immunosuppressive treatment with dexamethasone, 30 mice were orally infected with *C. parvum* oocysts on the same day. Two weeks after infection, mice shedding oocysts, identified by detecting oocysts in the stools using both Zeil Niessel staining and immunofluorescence, were pooled in three groups of 5 animals each. Two groups were treated with hydrochloride of the test compound dissolved in drinking water at a concentration of 5  $\mu\text{g/mL}$  (mice are expected to drink about 2 -5 mL of water per day), whereas the third group (control group) did not receive any treatment. To evaluate the efficacy of the treatment, the shedding of oocysts was monitored for 4 weeks. Animals receiving treatment showed a significant reduction in oocyst shedding after 1 week, and

oocysts could not be detected after 2 weeks of treatment with the test compound, and remained parasite free until the end of the experiment (4 weeks).

### Example 3

10 male Sprague-Dawley rats weighing 320 - 390 g were used with the scope to  
5 determine plasma levels of the compound of formula (I, R = -COONH<sub>2</sub>, A = -  
CONHCH<sub>2</sub>CH<sub>2</sub>-, R<sub>1</sub> = -C(=NH)-NH<sub>2</sub>) after intravenous and oral administration,  
respectively. Rats were anesthetized and the right jugular vein was cannulated and the  
cannula was left exposed on the neck to allow for drug administration and blood collection.  
Rats were administered 24 - 48 hours after recovery from surgical anesthesia.

10 Intravenous drug administration (10 mg/kg). 10 mg of hydrochloride of the test  
compound were dissolved in a vehicle constituted of DMSO and saline buffer (1 : 20 v/v)  
at a concentration of 1.5 mg/mL. The administered volume was 2 mL/kg as bolus.

Oral administration (20 mg/kg). 20 mg of hydrochloride of the test compound were  
dissolved in a vehicle constituted of DMSO and saline buffer (1 : 250 v/v) at a  
15 concentration of 5 mg/mL. The administered volume was 5 mL/kg.

Sample collection. Blood samples (400-500 µl) were withdrawn from jugular vein at  
intervals of 0, 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 680 and 1440 minutes after  
intravenous and oral administration, respectively, of test drug. The amount of collected  
blood was replaced by an equal volume of saline.

20 Plasma levels. The determination of test compound blood levels was performed by  
using a validated HPLC method. The limit of quantitation of the assay was 0.1 µg/0.5 mL.

After a single intravenous administration of 10 mg/kg of the drug, the mean drug  
concentration in blood declined according to a biexponential profile.

After a single oral administration of 20 mg/kg of the drug, no detectable amount of the drug was found in blood in the time interval 0 - 1440 minutes, indicating that the test compound is not orally adsorbed. Data are set forth in Table 1.

Table 1

5

min	i.v.		( $\mu\text{g/ml}$ )	p.o.
0	Nd			nd
5	10.0	$\pm$	0.95	nd
15	8.2	$\pm$	0.98	nd
30	6.9	$\pm$	0.67	nd
60	5.2	$\pm$	0.65	nd
90	3.8	$\pm$	0.59	nd
120	2.7	$\pm$	0.74	nd
180	1.5	$\pm$	0.44	nd
240	1.1	$\pm$	0.21	nd
360	0.89	$\pm$	0.15	nd
480	0.72	$\pm$	0.09	nd
680	0.53	$\pm$	0.16	nd
1440	0.27	$\pm$	0.11	nd

#### Example 4

0.15 mmol of hydrochloride of a compound of formula (I) and 0.38 mmol of  $\beta$ -cyclodextrin are dissolved in 100 mL of water with stirring for 6 hours. The resulting

product is separated by precipitation by cooling the solution at 3°C or, alternatively, by freeze-drying the solution.

Example 5

2,64 mmol of  $\beta$ -cyclodextrin are dissolved in 100 mL of water by applying a gentle  
5 heating. To the resulting solution there are added 1,06 mmol of hydrochloride of a compound of formula (I) with stirring for 6 - 12 hours. The product is separated by precipitation by cooling the solution at 3°C or, alternatively, by freeze-drying the solution.

Example 6

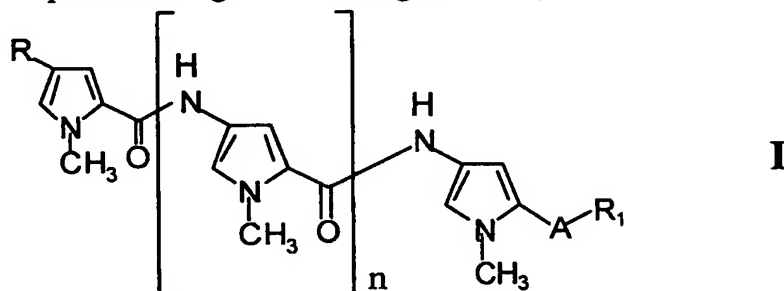
Mixtures of hydrochloride of a compound of formula (I) and of different amounts of  
10  $\beta$ -cyclodextrin, or 2-hydroxypropyl- $\beta$ -cyclodextrin, or  $\gamma$ -cyclodextrin are prepared by grinding, followed by sieving (<0.375 mm) and 10 minute of mixing in a turbo-mixer.

The disclosures in Italian Patent Application No. MI2003A001015 from which this application claims priority are incorporated herein by reference.



## CLAIMS

1. Use of a compound having the following formula (I):



wherein:

- 5 n is 0 or an integer comprised between 1 and 5;

R is a group  $R_2-X-C(=Z)-NH-$ , in which X represents a simple chemical bond, an aromatic or heteroaromatic radical, Z represents an oxygen atom or the NH group; and:

- 10 if X is a simple chemical bond,  $R_2$  is an oxygen atom, an alkyl, dialkylaminoalkyl, alkenyl, cycloalkyl, arylalkyl, arylalkenyl, haloalkyl, or an aromatic or heteroaromatic radical;  
if X is an aromatic or heteroaromatic radical,  $R_2$  is nitro, amino or formylamino;

or:

- 15 R is a group  $R_3-C(=Z)-$ , in which Z represents an oxygen atom or the NH group, and  $R_3$  represents a hydrogen atom, the  $-OR_4$  or  $-NR_5R_6$  group, and where:

$R_4$  is chosen from the group consisting of a hydrogen atom, an alkyl, cycloalkyl, arylalkyl, or an aromatic radical;

- 20  $R_5$  and  $R_6$ , either the same or different, are chosen from the group consisting of a hydrogen atom, an alkyl, cycloalkyl, arylalkyl, aromatic or

heterocyclic radical, optionally substituted with a formylamino or a carbamoyl group; or

R<sub>5</sub> and R<sub>6</sub>, joined together form an alkylene group, or the group -(CH<sub>2</sub>)<sub>2</sub>-O-(CH<sub>2</sub>)<sub>2</sub>- or the group -(CH<sub>2</sub>)<sub>2</sub>-NH-(CH<sub>2</sub>)<sub>2</sub>-;

5 A represents a simple chemical bond or the group -CO-NH-Y-, wherein Y is an alkylene or aromatic radical;

R<sub>1</sub> is chosen from the group consisting of -COOR<sub>4</sub>, -B-NR<sub>5</sub>R<sub>6</sub>, -C(=NH)-NH<sub>2</sub>, a heterocyclic radical containing nitrogen, wherein:

10 R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are as defined above, B represents a simple chemical bond or the -C=O group, and:

when R<sub>1</sub> is -B-NR<sub>5</sub>R<sub>6</sub>, and B is a simple chemical bond, or when R<sub>1</sub> is a heterocyclic radical, A is not a chemical bond;

or a pharmaceutical acceptable salt thereof in the manufacture of a pharmaceutical composition having activity against endoparasitosis in animals.

15 2. Use according to Claim 1, where the compound of formula (I) is chosen between distamycin and a compound of formula (I) wherein:

n is as previously defined;

R is the -CONH<sub>2</sub> group, A is the -CONHCH<sub>2</sub>CH<sub>2</sub>- group, R<sub>1</sub> is the -C(=NH)-NH<sub>2</sub> group or the -CH<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub> group;

20 R is the -NH-CH(=NH) group, A is the -CONHCH<sub>2</sub>CH<sub>2</sub>- group, R<sub>1</sub> is the -C(=NH)-NH<sub>2</sub> group or the -CH<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub> group or the -CONH<sub>2</sub> group;

and the pharmaceutically acceptable salts thereof.

3. Use according to Claim 1, where the pharmaceutical composition is for oral use.

4. Use according to Claim 1, where the pharmaceutical composition is for the prophylaxis and/or treatment of endoparasitosis in animals.

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5. Use according to Claim 4, where the endoparasitosis is chosen from Trichomoniasis, Giardiasis, Istomoniasis, Amoebiasis, Coccidiosis, and Balantidiosis.
6. Use of a compound of formula (I), or a pharmaceutically acceptable salt thereof, in association or combination with a cyclodextrin in the manufacture of a veterinary pharmaceutical composition having activity against endoparasitosis in animals.
- 5 7. Use according to Claim 6, where the pharmaceutical composition is for oral use.
8. Use according to Claim 6, where the pharmaceutical composition is for the prophylaxis and/or treatment of endoparasitosis in animals.

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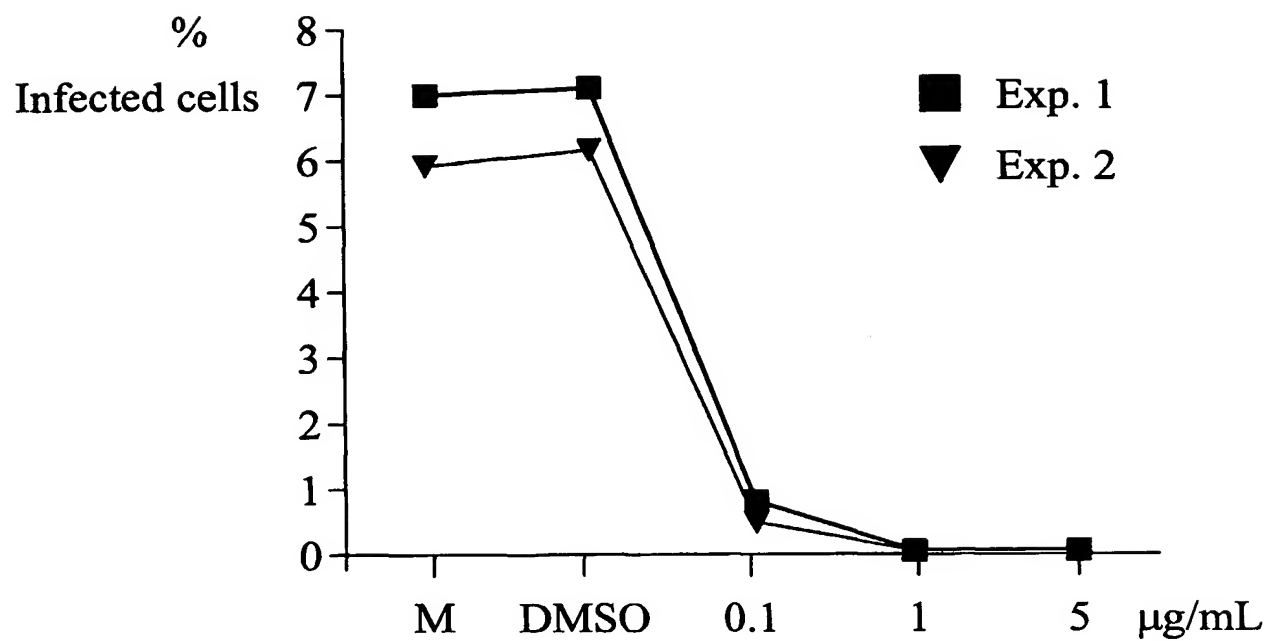


Figure 1

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP2004/005157

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/4025 A61K47/40 A61P33/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, FSTA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 670 534 A (ARCAMONE FEDERICO ET AL) 23 September 1997 (1997-09-23) column 1, line 5 - column 2, line 27 column 3, lines 1-10 column 10, lines 41,42; claim 5; example 1 -----	1-8
Y	US 5 712 260 A (BUZZI GIOVANNI ET AL) 27 January 1998 (1998-01-27) cited in the application column 1, lines 5-8,44-50 column 2, line 54 - column 3, line 19 -----	1-8
A	US 5 472 976 A (ANIMATI FABIO ET AL) 5 December 1995 (1995-12-05) cited in the application column 1, lines 30-64 column 7, lines 1-12; example 1 ----- -/-	1-8



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
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- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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Date of the actual completion of the international search

15 September 2004

Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP2004/005157

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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P,X	<p>WO 2004/004691 A (ARCAMONE FEDERICO ; CITERNESI UGO RAFFAELLO (IT)) 15 January 2004 (2004-01-15) page 1, lines 1-18 page 2, line 16 page 4 page 6, line 6 - page 7, line 1 -----</p>	1-8

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information on patent family members

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